Exposure to arsenic during pregnancy and newborn mitochondrial DNA copy number: A birth cohort study in Wuhan, China

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Declaration of interest : none.

Abbreviations: Al, aluminium; As, Arsenic; BMI, body mass index; CI, confidence interval; ICC, intraclass correlation coefficient; ICP-MS, inductively coupled plasma mass spectrometry; LOD, limit of detection; Mn, manganese; mtDNAcn, mitochondrial DNA copy number; NO_2, nitrogen dioxide; Pb, lead; qPCR, quantitative real-time polymerase chain reaction; SD, standard deviation; SG, specific gravity; Tl, thallium.

Abstract

Background: Arsenic (As) is a widely distributed environmental chemical with potentially different toxicities. However, little is known about the impact of maternal As exposure on newborn mitochondrial DNA copy number (mtDNAcn), which may lie on the pathway linking As exposure to adverse health impacts.

Objectives: We aimed to explore whether maternal As exposure was associated with newborn mtDNAcn.

Methods: We conducted a birth cohort study of 762 mother-infant pairs in Wuhan, China, 2013-2015. Cord blood mtDNAcn was determined using qPCR. Maternal urinary As levels in each trimester were quantified by ICP-MS. Multiple informant models were used to examine the associations of repeated urinary As levels with cord blood mtDNAcn.

Results: The median urinary As levels in the first, second, and third trimesters were 17.2 μg/L, 16.0 μg/L and 17.0 μg/L respectively. In the multivariate model, each doubling increase in the first-trimester urinary As level was associated with a 6.6% (95% CI: -12.4%, -0.5%) decrease in cord blood mtDNAcn. The highest versus lowest quintile of first-trimester urinary As level was related to a 19.0% (95% CI: -32.9%, -2.2%) lower cord blood mtDNAcn. There was significant association of urinary As levels in the second and third trimesters with cord blood mtDNAcn. The inverse relationship between first-trimester urinary As level and cord blood mtDNAcn was more pronounced among female infants.

Conclusions: First-trimester As exposure was associated with decreased cord blood mtDNAcn. The potential health impacts of decreased mtDNAcn in early life need to be further clarified.

Keywords: Arsenic; maternal exposure; newborns; mitochondrial DNA copy number;

trimester-specific

1. Introduction

Arsenic (As), a widely distributed metalloid element, is a naturally occurring element that exists in both organic and inorganic forms (ATSDR, 2007). The inorganic forms of As are harmful, while most of the organic forms of As are essentially harmless. Human are exposed to As mainly through drinking water and food (WHO, 2018). Upon ingestion, As is metabolized and mainly excreted through urine, and can be measured in urine, blood, or hair (ATSDR, 2007). As levels in urine can reflect ongoing exposures and are well correlated with As intake from food and drinking water (Ahsan et al., 2000; Calderon et al., 1999; Pellizzari and Clayton, 2006). It has been estimated that more than 200 million people worldwide might be chronically exposed to As in drinking water at levels above the World Health Organization (WHO) recommended limit of 10 μg/L (Naujokas et al., 2013). As-contaminated drinking water is widespread and represents a major public health problem worldwide (Kapaj et al., 2006; MM Rahman et al., 2009).

Exposure to As is of particular concern among pregnant women and fetuses because they are especially vulnerable to some environmental toxicants (Vahter, 2009). As can easily cross placenta and has been detected in cord blood (Concha et al., 1998; Hall et al., 2007). Extensive evidence suggested that As exposure during pregnancy was associated with adverse pregnancy and birth outcomes (e.g., spontaneous abortion, stillbirth, infant mortality, and fetal growth restriction) (Quansah et al., 2015). These impacts of maternal As exposure on adverse health outcomes have been suggested to result from the increased oxidative stress (Ahmed et al., 2011; A Rahman et al., 2009).

Mitochondria, intracellular organelles, are the primary target and major intracellular source of reactive oxygen species (ROS) in animal and human cells (Yakes and Van Houten, 1997). Each animal and human cell consists of several hundreds to a

thousand mitochondria, each carrying 2-10 copies of mitochondrial DNA (mtDNA). Compared with nuclear DNA, mtDNA has a high mutation rate and is more susceptible to ROS-induced damage due to lack of protective histone and limited repair capacity (Lee and Wei, 2000; Linnane et al., 1989). It has been reported that mitochondria can compensate for mtDNA oxidative damage by the alteration of mtDNA copy number (mtDNAcn) (Lee et al., 2000; Yakes and Van Houten, 1997), thus mtDNAcn has been considered as a marker of mitochondrial response to damage. MtDNAcn has been inversely associated with aging-related diseases, such chronic kidney disease (Tin et al., 2016), cardiovascular disease (Ashar et al., 2017), and all-cause mortality (Ashar et al., 2015).

Low mtDNAcn and mutations in mtDNA resulting from oxidative damage have been reported to persist and accumulate over time (Kujoth et al., 2005; Mengel-From et al., 2014; Sondheimer et al., 2011), indicating that early exposures may influence later mitochondrial health. In addition, decreased mtDNAcn was related to fetal outcomes that are critical predictors of health in later life like intrauterine growth restriction, birth weight, and birth length (Clemente et al., 2016; Clemente et al., 2017; Mando et al., 2014). Thus, identification of the relationships between early-life exposures and newborn mtDNAcn may be a major step forward in unravelling the early-life determinants of diseases in later life. A growing body of studies have been conducted to explore the determinants of newborn mtDNAcn, such as maternal smoking (Bouhours-Nouet et al., 2005), maternal lifetime stress (Brunst et al., 2017), maternal air pollution exposure (particulate matters with aerodynamic diameter $\leq$ 2.5 μm ($PM_{2.5}$) (Brunst et al., 2018; Rosa et al., 2017) and $\leq$ 10 μm (PM_{10}) (Janssen et al., 2012), nitrogen dioxide (NO_2) (Clemente et al., 2016; Clemente et al., 2017), and household air pollution (Kaali et al., 2018)), and maternal heavy metal exposure

(Kupsco et al., 2019; Liu et al., 2019; Sanchez-Guerra et al., 2019; Vriens et al., 2017; Wu et al., 2019; Xu et al., 2019). To date, only one study from Belgium showed a positive relationship of cord blood As with placental mtDNAcn (Vriens et al., 2017). Evidence regarding the relationships between trimester-specific As exposure and newborn mtDNAcn was lacking.

Therefore, we explored whether maternal As exposure during pregnancy was related to cord blood mtDNAcn and identified the sensitive exposure windows in a birth cohort study.

2. Material and methods

2.1. Study population

This birth cohort study was conducted between November 2013 and March 2015 at the Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital) in Wuhan city, Hubei province, China. Briefly, 762 mother-infant pairs were enrolled if the mothers met the following criteria: 1) residing in Wuhan City, 2) a singleton gestation with <16 weeks of pregnancy at enrollment, and 3) being willing to deliver at study hospital and planning to attend prenatal examination. We excluded 16 participants with missing cord blood samples or ineligible DNA quality, 746 mother-infant pairs were included for the final analysis. Of the 746 included pregnant women, 598 (80.2%) provided urine samples at all three trimesters. The number of women with urine samples in the first, second, and third trimester were 746, 745, and 599, respectively.

The study was approved by the ethics committees of Tongji Medical College, Huazhong University of Science and Technology and the Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital). All participants signed informed consent.

2.2. Urine collection and exposure measurements

Spot urine samples were collected from pregnant women in the first (mean ± standard deviation (SD), 13.0 ± 1.1 weeks), second (23.6 ± 3.2 weeks), and third trimesters (35.1 ± 3.1 weeks). Urine samples were collected in polypropylene tubes and were frozen at -20 °C until analyses.

Maternal As exposures were estimated using urinary As levels. Total urinary As levels (including organic and inorganic As) were measured using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700, Agilent Technologies). Urinary levels of lead (Pb), aluminium (Al), manganese (Mn), and thallium (Tl) were also quantified by ICP-MS, because they were reported to be associated with newborn mtDNAcn (Kupsco et al., 2019; Liu et al., 2019; Sanchez-Guerra et al., 2019; Wu et al., 2019). The detailed methods of measurement and quality control have been described previously (Liu et al. 2018). In brief, urine samples were thawed at room temperature and then nitrated overnight by 3% HNO_3. The resulting samples were digested by ultrasound at 40 °C for 1h. The limits of detection (LOD) for urinary As, Pb, Al, Mn, and Tl were 0.020 μg/L, 0.008 μg/L, 0.106 μg/L, 0.050 μg/L, and 0.020 μg/L, respectively. One urinary arsenic concentration in this study was below the LOD, which was replaced as LOD/$\sqrt{2}$. The intra-day and inter-day coefficients of variation for urinary As, Pb, Al, Mn, and Tl were 0.286%-0.858% and 0.272%-2.584%, respectively.

Urinary specific gravity (SG) was measured by a refractometer (Atago PAL-10S; Atago, Tokyo, Japan). Levels of urinary As and other metals (Pb, Al, Mn, and Tl) were corrected to control for variations in urine dilution by SG according to the following formula: $P_{SG} = P[(1.012-1)/(SG-1)]$, where P_{SG} is the SG-corrected exposure levels, P is the measured exposure levels, the value of 1.012 is the median

SG in this study population, and SG is the specific gravity of the individual urine samples.

2.3. Measurements of mtDNAcn

Cord blood was collected immediately at delivery. Blood samples were centrifuged and placed at -80 °C until DNA extraction. DNA was isolated from the leukocytes of umbilical cord blood samples by Wizard® Genomic DNA Purification (Promega Corporation, Madison, WI, USA). Relative cord blood mtDNAcn was measured by quantitative real-time PCR (qPCR) assay and the sequences of primer, reaction mixture, and PCR thermal cycling profile are described previously (Liu et al., 2019; Wu et al., 2019). In brief, relative cord blood mtDNAcn was calculated by the ratio of the mitochondrial gene copy numbers (*mtND1*) to the single-copy nuclear control gene [human beta-globin (*hbg*)]. All measurements were conducted in triplicates using the ViiA™ 7 Dx Real-Time PCR System (Applied Biosystems) in 384-well plates. A pool of 50 genomic DNA samples selected randomly from our study population was used to construct a standard curve with five-point serial dilution, ranging from 104 ng/μL to 0.4 ng/μL ($R^2 \geq 0.99$).To ensure quality control, each plate included standard curve, negative controls, and inter-plate controls. The intra-run and inter-run coefficients of variation for mtDNAcn measurements were 2.8% and 3.8%, respectively.

2.4. Covariates

Information on socio-demographic characteristics and lifestyle behaviors during pregnancy was collected via standard questionnaires, including maternal age, education, occupation, alcohol consumption during pregnancy, and active and passive smoking during pregnancy. Information on parity, infant sex, and birth date was obtained from medical records. Season of birth was categorized into warm period

(June-November) and cold period (December-May). Gestational age at birth was determined based on the last menstrual period. Pre-pregnancy body mass index (BMI) was estimated according to the ratio of pre-pregnancy weight (kg) to height squared (m^2).

2.5. Statistical analysis

Continuous data were shown as mean ± SD (normally distributed) or median with 25-75th (not normally distributed), and categorical data as number (frequency). Urinary As levels and mtDNAcn were ln-transformed to improve the normality. We computed Spearman correlation coefficients of uncorrected and SG-corrected urinary As levels in three trimesters. Reproducibility of urinary As levels in three trimesters was estimated using the intraclass correlation coefficient (ICC). The ICC was determined by dividing the between-person variance by the sum of within- and between-person variances. The ICC values of < 0.40, 0.40-0.75, and > 0.75 were defined as weak, moderate, and strong reproducibility, respectively (Rosner, 2011).

Multiple informant models (Sanchez et al., 2011) were applied to examine the trimester-specific relationships between urinary As levels and cord blood mtDNAcn. The multiple informant models not only simultaneously assessed the association between As exposure in each trimester and cord blood mtDNAcn in the same model, but also tested homogeneity in relationships of maternal As exposure with cord blood mtDNAcn across three trimesters. We assessed the association of maternal As exposure with cord blood mtDNAcn in two ways: continuous (ln-transformed maternal As level) and categorical (quintiles of maternal As level) variables. *P*-values for trend were determined by fitting the median value of each quintile as a continuous variable. Covariates were chosen based on existing studies or they could lead to changes of main effect by >10% (Greenland, 1989). Multivariate models were

adjusted for maternal age (continuous), pre-pregnancy BMI (continuous), education (junior high school or below/high school/college or above), parity (primiparous/ multiparous), occupation (employed/unemployed), passive smoking (yes/no), infant sex (male/female), and gestational age (continuous). To improve interpretability of regression analyses consisting of ln-transformed exposure and/or outcome variables, we calculated the percent change and 95% confidence interval (CI) in cord blood mtDNAcn for a doubling or quintile of urinary As.

Given that previous studies reported gender differences in the effects of As exposure (Gilbert-Diamond et al., 2016; Kippler et al., 2012), we performed stratified analysis based on infant sex. Tests for interactions were performed using the Wald test (Kaufman and MacLehose, 2013). We also conducted a series of sensitivity analyses: additional adjustment for month of birth, season of birth, and gestational age at urine collection individually; additional adjustment for other metals (Pb, Al, Mn, and Tl); excluding mothers aged ≥ 35 years (advanced maternal age). To explore the possible misclassification induced by the missing urine samples, we also repeated our regression models by restricting to participants with all three urine samples.

All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC). A two-tailed *P*-value < 0.05 was considered significant.

3. Results

Characteristics of 746 mother-infants pairs are displayed in Table 1. Mean pre-pregnancy BMI was 20.8 ± 2.8 kg/m^2 and mean maternal age was 28.6 ± 3.3 years. Among the mothers, 589 (79.0%) had higher education attainment, 446 (59.8%) were employed, 244 (32.7%) were passively exposed to cigarette smoking, and 644 (86.3%) were primiparous. The newborns (383 males and 363 females), including 18 (2.4%) preterm infants, had a mean gestational age at birth of 39.4 ± 1.2 weeks. The

characteristics of participants did not differ significantly between mothers with urine samples in all three trimesters and those with missing urine sample, except for education, passive smoking during pregnancy, and season of birth (Table S1).

Table 2 presents the distributions, reproducibility (ICCs), and Spearman correlation coefficients of the uncorrected and SG-corrected urinary As levels across three trimesters. The median (25th-75th percentile) levels of SG-corrected urinary As were 17.2 (12.4-25.6) μg/L for the first trimester, 16.0 (11.7-24.3) μg/L for the second trimester, and 17.0 (12.2-24.1) μg/L for the third trimester. The SG-corrected ICC of urinary As levels across three trimesters was 0.16, suggesting a week reproducibility. The Spearman's correlation coefficients among urinary As levels during three trimesters ranged from 0.15 to 0.19. The distributions of urinary Pb, Al, Mn, and Tl levels in three trimesters are shown in Table S2. Spearman correlation coefficients of urinary levels of As and other metals in three trimesters are shown in Table S3.

Table 3 shows the assoication of maternal urinary As level with cord blood mtDNAcn. In the unadjusted model, a doubling increase in the first-trimester urinary As level was associated with a 6.6% (95% CI: -12.5%, -0.4%) decrease in cord blood mtDNAcn. After adjustment for potential confounding factors, the association was not materially changed, equivalent to a 6.6% (95% CI: -12.4%, -0.5%) decrease in cord blood mtDNAcn for each doubling increase in the first-trimester urinary As level. The trimester-specific relationships between maternal urinary As levels and cord blood TL were found (P_{int}= 0.044). We also assessed the association between quintiles of maternal urinary As level and cord blood mtDNAcn (Fig. 1 and Table S4). Compared to the lowest quintile, the highest quintile of the first-trimester urinary As level had a 19.0% (95% CI: -32.9%, -2.2%) decrease in cord blood mtDNAcn, with a significant dose-response relationship across these quintiles (*P* for trend = 0.041). No

significant associations between urinary As levels in the second and third trimesters and cord blood mtDNAcn were observed.

In analysis stratified by infant sex (Table 4), first-trimester urinary As level was inversely related to cord blood mtDNAcn (percent change, -10.4%; 95% CI, -19.0%, -1.0%) among female infants, but not male infants (percent change, -3.2%; 95% CI, -10.7%, 5.1%). In the sensitivity analyses, the inverse association between first-trimester urinary As level and cord blood mtDNAcn was not materially changed with further adjustment for month of birth, season of birth, and gestational age at urine collection individually; additional adjustment for other metals (Pb, Al, Mn, and Tl); excluding mothers aged ≥ 35 years; or restricting the analyses to mothers with all three urine samples (Table S5).

4. Discussion

To our knowledge, this is the first report to explore the effects of trimester-specific As exposure on cord blood mtDNAcn. We found that first-trimester As exposure was related to decreased cord blood mtDNAcn, particularly among female infants. No significant associations between maternal As exposure in the second and third trimesters and cord blood mtDNAcn were observed.

Almost all of the pregnant women had detectable urinary As levels, suggesting that our study participants were widely exposed to this metalloid element. Urinary As levels in our study (median, 16.6 μg/L) were higher than those reported among pregnant women from the United States (median, 3.4-4.3 μg/L) (Farzan et al. 2016; Gilbert-Diamond et al. 2016; Gossai et al. 2015) and Canada (Thomas et al. 2015), and were lower than those reported from the Bangladesh (median, 81-94 μg/L) (Rahman et al. 2011; Tofail et al. 2009), Japan (geometric mean, 76.9 μg/g creatinine) (Shirai et al. 2010), Mexico (median, 23.3 μg/L) (Laine et al. 2015), and Chile

(median, 30.3-61.7 μg/g creatinine) (Hopenhayn et al. 2003). In comparison with general population, our study population had higher As levels than those reported from the United States (median, 7.5 and 8.7 μg/g creatinine for men and women) (Kuo et al. 2015), and had lower As levels than those reported from the Spain (median, 52.1 μg/g creatinine) (Navarro Serrano et al. 2016) and Bangladeshi (median, 257 μg/g creatinine) (Howe et al. 2016). The difference in urinary As levels in different regions were possibly due to the variations in food intake, lifestyle factors, and environmental contamination. As has a short biological half-life in urine and is rapidly metabolized (ATSDR, 2007). However, as far as we know, no studies have reported the temporal variability in urinary As levels over pregnancy. In our study, we observed that the ICC for urinary As levels was 0.16, suggesting a week reproducibility during pregnancy. The week reproducibility was possibly due to the biochemical or physiological changes related to progression of pregnancy (Abduljalil et al., 2012) or the change in external exposure level of As.

Drinking water and diet are two major routes for As exposure among general population (WHO, 2018). Our participants were recruited in Wuhan, an inland city in China. All participant are urban residents of Wuhan city and use the municipal tap water as the source of drinking water with lower arsenic level (1-3 mg/L) (Sun et al. 2017), which is below the WHO safety standard of 10 μg/L (WHO, 2011). In addition, the staple food of residents is rice in Wuhan city. Rice consumption has been reported to be related to urinary As levels among pregnant women and general population (Gilbert-Diamond et al., 2011; Islam et al., 2016).

Evidence on maternal exposure to environmental pollutants associated with newborn mtDNAcn has begun to accumulate, but the direction of effects from different pollutants varied. Studies reported increased newborn mtDNAcn in

association with maternal exposure to lead (Sanchez-Guerra et al., 2019), aluminum (Liu et al., 2019), and manganese (Kupsco et al., 2019). Decreased newborn mtDNAcn was reported in relation to maternal smoking (Bouhours-Nouet et al., 2005), maternal thallium exposure (Vriens et al., 2017; Wu et al., 2019), and maternal air pollution exposure [$PM_{2.5}$ (Brunst et al., 2018; Rosa et al., 2017), PM_{10} (Janssen et al., 2012), NO_2 (Clemente et al., 2016; Clemente et al., 2017), and household air pollution (Kaali et al., 2018)]. The variation in the association of environmental exposures with newborn mtDNAcn is possibly due to variations in exposure level, type of exposure, exposure duration, and exposure population. However, few studies have examined the association of maternal As exposure with newborn mtDNAcn. Contrary to our findings, a research of 233 mother-infant pairs performed by Vriens et al. showed that cord blood As level was related to increased placental mtDNAcn (Vriens et al., 2017). The difference in As level may be the reason for the inconsistent findings. Urinary As level in Vriens's study was much lower than that in our study (mean, 1.19 μg/L vs 22.9 μg/L). Another possible reason was the different timing of As measurements. Urinary As levels in three trimesters were assessed in our study, while Vriens's study assessed As level using cord blood collected after delivery, representing the short-term neonatal exposure.

Although the underlying mechanisms by which maternal As exposure can lead to decreased cord blood mtDNAcn are not fully understood, one plausible explanation is the generation of oxidative stress induced by As exposure (Ahmed et al., 2011; Jomova et al., 2011). Compared with nuclear DNA, mtDNA is especially prone to oxidative damage due to lack of protective histone and lower repair capacity (Lee and Wei, 2000; Linnane et al., 1989). Mitochondria can respond to mtDNA oxidative damage by increasing mtDNAcn (Lee and Wei, 2005). However, with increasing

356 mtDNA oxidative damage, the compensatory mechanism may be deficiency, leading
357 to decrease in mtDNAcn (Lee and Wei, 2005). Furthermore, mitochondria are mainly
358 responsible for cellular energy production (ATP levels). MtDNAcn is related to the
359 size and number of mitochondria (Lee and Wei, 2005), which can alter under different
360 energy demands. Experimental studies have reported that exposure to As was related
361 to reduced Ca^{2+}-ATPase activity (Majumdar et al., 2011; Muthumani and Miltonprabu,
362 2015), which led to decrease in energy production. Increases in energy demands can
363 overwhelm the mitochondria and result in decreased mtDNAcn.
364 In the present study, we observed that first-trimester As exposure was associated
365 with decreased cord blood mtDNAcn, suggesting that mtDNAcn may be susceptible
366 to As exposure during the early developmental stages of fetus. Early pregnancy is
367 generally a critical period to environmental exposures, and the developing fetus is
368 susceptible to the oxidative stress. Once the utero-placental circulation is established,
369 the fetus becomes more resistant to oxidative stress by increasing antioxidant defenses
370 (Dennery, 2007), which can protect the mitochondria.
371 Interestingly, we found that the effect of maternal As exposure in the first trimester
372 on cord blood mtDNAcn was female-specific. Although the potential mechanisms are
373 yet to be elucidated, it has been recently reported that increased AQP9 gene
374 expression may lead to increased As transport in female fetal placenta but not in male,
375 suggesting that AQP9 gene may be upregulated in response to As in a female
376 sex-specific manner (Winterbottom et al., 2017).
377 Our study has several strengths, including large sample size, prospective design,
378 and detailed covariates. In addition, we measured repeated maternal urinary As levels,
379 which helped to clarify the effect of maternal As exposure on newborn mtDNAcn
380 with more precision. Moreover, multiple informant models were applied to examine

the relationship of maternal As exposure with newborn mtDNAcn, which enabled us to identify the sensitive windows of maternal As exposure for newborn mtDNAcn.

Our study has limitations. First, we did not distinguish different As species or metabolites in urine samples, and therefore our results represent the effects of both inorganic and organic As exposures. Second, although we carefully adjusted for several potential confounders, it is not possible to entirely rule out the possibility of residual confounding by unobserved factors in relation to As exposure and mtDNAcn. Third, all study population in the current study were Chinese, which may limit the generalizability of our findings.

5. Conclusion

Our study provided evidence that maternal As exposure in the first trimester was related to decreased newborn mtDNAcn, which suggests a sensitive window for maternal As exposure. Future investigations are needed to further explore the effects of As-related decrease in mtDNAcn at birth on subsequent health of offspring.

Conflict of interest

The authors declare they have no actual or potential competing financial interests.

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Table 1. Characteristics of mother-infant pairs (n=746).

Variables	Mean ± SD, geometric mean (25th-75th percentile) or n (%)
Maternal characteristics	
Maternal age (years)	28.6 ± 3.3
Pre-pregnancy BMI (kg/m^2)	20.8 ± 2.8
Education	
Junior high school or below	42 (5.6)
high school	115 (15.4)
College or above	589(79.0)
Occupation	
Employed	446 (59.8)
Unemployed	294 (39.4)
Missing	6 (0.8)
Alcohol use during pregnancy	
Yes	0 (0.0)
No	746 (100.0)
Smoking during pregnancy	
Yes	0 (0.0)
No	746 (100.0)
Passive smoking during pregnancy	
Yes	244 (32.7)
No	502 (67.3)
Parity	
Primiparous	644 (86.3)
Multiparous	102 (13.7)
Infant characteristics	
Infant sex	
Male	383 (51.3)
Female	363 (48.7)
Gestational age (weeks)	39.4 ± 1.2
Preterm birth (< 37 weeks)	
Yes	18 (2.4)

No	728 (97.6)
Season of birth	
Warm period (June-November)	393 (52.7)
Cold period (June-November)	353 (47.3)
Cord blood mtDNAcn	1.2 (0.7–2.2)

Abbreviations: BMI, body mass index; mtDNAcn, mitochondrial DNA copy number. Continuous variables are presented by mean ± SD (normally distributed) or geometric mean with 25-75th percentile (not normally distributed); categorical variables are expressed by n (%).

Table 2. Distributions and intraclass correlation coefficients, and spearman correlation coefficients of maternal urinary arsenic levels across three trimesters.

Arsenic levels	GM (95% CI)	Percentile			1st trimester	2nd trimester	3rd trimester	ICC
		25th	50th	75th				
Uncorrected (μg/L)								0.16
1st trimester	16.6 (15.5, 17.7)	9.6	17.8	30.0	1.00			
2nd trimester	13.8 (12.9, 14.7)	7.6	13.4	24.9	0.17	1.00		
3rd trimester	13.2 (12.4, 14.1)	7.6	12.7	22.9	0.16	0.15	1.00	
SG-corrected (μg/L)								0.16
1st trimester	18.3 (17.5, 19.2)	12.4	17.2	25.6	1.00			
2nd trimester	17.4 (16.6, 18.2)	11.7	16.0	24.3	0.18	1.00		
3rd trimester	18.0 (17.1, 18.9)	12.2	17.0	24.1	0.19	0.16	1.00	

Abbreviations: CI, confidence interval; GM, geometric mean; ICC, intraclass correlation coefficient; SG, specific gravity.

Table 3. Associations between maternal arsenic exposure during pregnancy and cord blood mtDNAcn.

Arsenic concentrations (μg/L)	No. of subjects	Percent change (95% CI)	
		Model 1	Model 2
1st trimester	746	-6.6 (-12.5, -0.4)	-6.6 (-12.4, -0.5)
2nd trimester	745	3.3 (-3.3, 10.4)	2.4 (-4.0, 9.3)
3rd trimester	599	4.2 (-4.7, 13.8)	4.2 (-4.7, 13.9)
P_{int}[a]		0.035	0.044

Abbreviations: CI, confidence interval.

Model 1: unadjusted

Model 2: adjusted for maternal age, pre-pregnancy BMI, parity, education, occupation, passive smoking during pregnancy, infant sex, and gestational age.

[a] Score test of homogeneity of regression coefficients across three trimesters.

Table 4. Associations between maternal arsenic exposure during pregnancy and cord blood mtDNAcn, stratified by infant sex.

Arsenic levels (μg/L)	Percent change (95% CI)		$P_{sex\text{-int}}$ [b]
	Model 1	Model 2	
Infant sex			
Male (n=383)			
1st trimester	-3.7 (-11.5, 4.8)	-3.2 (-10.7, 5.1)	0.237
2nd trimester	-0.6 (-10.1, 9.8)	-1.9 (-11.0, 8.2)	0.227
3rd trimester	5.2 (-7.3, 19.5)	3.2 (-9.1, 17.2)	0.814
P_{int} [a]	0.510	0.615	
Female (n=363)			
1st trimester	-10.1 (-18.9, -0.5)	-10.4 (-19.0, -1.0)	
2nd trimester	7.3 (-2.0, 17.4)	6.6 (-2.8, 16.8)	
3rd trimester	2.9 (-8.6, 15.9)	5.4 (-6.5, 18.7)	
P_{int} [a]	0.010	0.006	

Abbreviations: CI, confidence interval.

Model 1: unadjusted

Model 2: adjusted for maternal age, pre-pregnancy BMI, parity, education, occupation, passive smoking during pregnancy, and gestational age.

[a] Score test of homogeneity of regression coefficients across three trimesters.

[b] *P*-value for the interaction between infant sex and maternal arsenic exposure within each trimester.